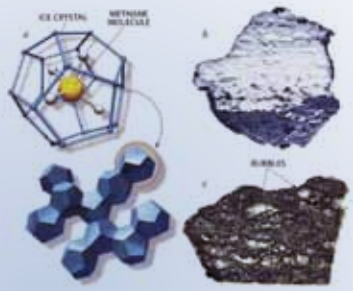
*Our focus is on developing and using advanced metabolomic techniques to study C-1 prokaryotes. Our definition of "C-1" includes a variety of prokaryotic metabolic systems that involve the transformation of single-carbon compounds. We have targeted specific C-1 metabolic processes of interest to DOE.*



# Microbial Metabolic Systems

## Description

The Microbial Metabolic Systems focus at INL is a systems biology approach to more effectively understanding and controlling microbial processes. An enhanced understanding of key microbial processes is being gained by coupling existing genomics, transcriptomics, and proteomics efforts with new metabolomic techniques and data. We use hypothesis-driven research to investigate the impacts of environment, perturbations and manipulations on microbial systems for the purpose of controlling the products and applications of those systems.

Our focus is on developing and using advanced metabolomic techniques to study C-1 prokaryotes. Our definition of "C-1" includes a variety of prokaryotic metabolic systems that involve the transformation of single-carbon compounds. We have targeted specific C-1 metabolic processes of interest to DOE:

- Methanogenesis – methane production by methanogenic bacteria
- Methanotrophy – methane/methanol utilization by methanotrophic bacteria
- Bioleaching – carbon fixation in chemoautolithotrophic bacteria and archaea (e.g., *Acidithiobacillus ferrooxidans, Acidianus spp.*, etc.)
- Calcite Precipitation – subsurface calcite precipitation by urea hydrolyzing bacteria
- Bicarbonate Transport – photoautotrophic carbon fixation by cyanobacteria
- Hydrogenase Systems – hydrogen production by *Carboxydothermus hydrogenoformans*.

INL is leveraging existing research programs and expertise in C-1 microbial metabolic systems to develop a recognized capability that will be more broadly applied to other microbial systems relevant to DOE missions.

## Rationale

The initial research directions chosen for this initiative are founded on the following facts:

- C-1 bioprocesses play a crucial role in many of the problems and opportunities faced by DOE
- INL has established C-1

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research strengths in:
  - Chemolithotrophy (biomining, methanogens, and hydrates),
  - Biogeochemistry (calcite precipitation), and
  - Heterotrophy (methanotrophs)
- INL has invested in current C-1 research themes in photoautotrophy (bicarbonate sequestration)
- INL has experience in moving research results from the conceptual level in the lab to the field and associated applications.

"Use-inspired" C-1 research examples:

- Analyze differential gene expression to identify key parameters controlling bioleaching of copper in ores and concentrates
- Coordinate analyses of gene expression in *Acidithiobacillus* with proteomics to understand the influence of environmental parameters on the physiology of these acidophilic microorganisms and therefore control the liberation of valuable metals from sulfide minerals
- Develop methods to identify and quantify proteins/metabolites differentially expressed by a microbial community when urea is added to the system, and relate expression patterns to urea hydrolysis, calcite precipitation and thereby control metal co-precipitation
- Determine environmental conditions that affect bicarbonate transport in order to fine-tune this potentially rate-limiting step in $CO_2$ fixation
- Conduct directed evolution of key rate-limiting enzymes to improve function and genetically modify the whole organism to improve $CO_2$ fixation rates by altering enzyme expression at rate-limiting steps
- Use genomics and proteomics to detect and extract diverse hydrogenases in habitats where hydrogen governs microbial success, and then use these hydrogenases to fabricate prototype storage materials
- Apply novel expression vectors to create enzymes of industrial value.

## Status

- Internally funded research program to metabolically engineer extremophilic microorganisms for applications in bioenergy
- Internally funded research program to improve understanding of hydrogenase systems in *Carboxydothermus hydrogenoformans*
- Internally funded research effort investigating environmental parameters on bicarbonate transporters in cyanobacteria
- Continuing investment in professional development of staff and acquisition of laboratory equipment (microarray, mass spectrometer, MALDI source, etc.) in support of these research programs
- Leveraging ongoing basic and applied research programs for DOE-FE, EERE, SC (OBER) and DHS to support new research thrusts, and
- Forming strategic partnerships, for example:
  - University of Maryland
  - Montana State University, Thermal Biology Institute
  - Pacific Northwest National Laboratory
  - University of Chile.

## For more information

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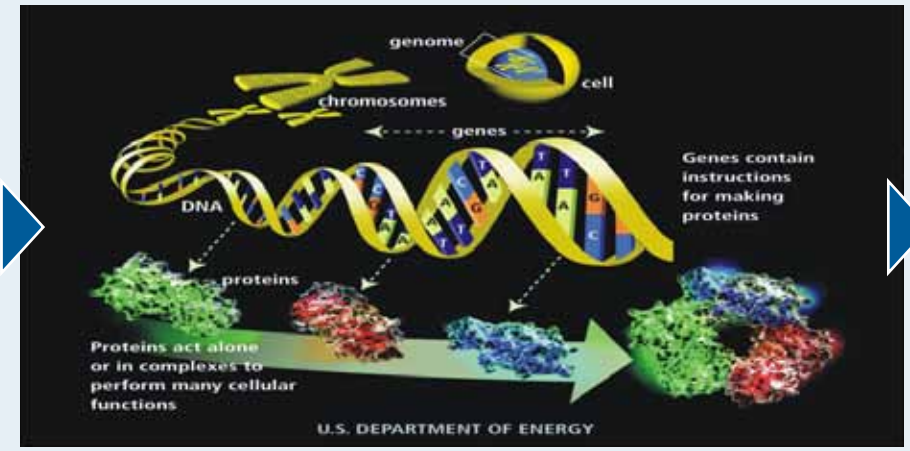
### Inputs

- Environmental variables
- Perturbations
- Genetic manipulations




### Products and Applications

- Biomining
- Remediation
- $CO_2$ sequestration
- Energy efficiency
- Chemicals of value

***We use hypothesis-driven research to investigate the impacts of environment, perturbations and manipulations on microbial systems for the purpose of controlling the products and applications of those systems. (Illustration adapted from DOE-SC, Genomics: GtL Web page.)***